Supporting Information

Transistor-Like Behavior of Single Metalloprotein Junctions

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1. Experimental

Sample Preparation

Reported protocols were used to prepare atomically flat gold surfaces[1] and to attach azurin on gold[2] through native cysteines C3 and C26, which results in a defined orientation of the protein on the surface, while preserving its native-like conformation[3] and electrochemical properties.[4, 5] Azurin and all reagents were purchased from Sigma. Nonredox, zinc-substituted azurin for control experiments was obtained as described.[6, 7]

Single-Protein Junctions in electrochemical environment

All experiments were performed with a PicoSPM microscope head and a PicoStat bipotentiostat (Molecular Imaging) controlled by Dulcinea electronics (Nanotec Electronica) using WSxM 4.0 software.[8] A homemade electrochemical cell was used in four-electrode configuration, using a Pt:Ir (80:20) wire as counter electrode and a miniaturized ultralow leakage membrane Ag/AgCl (SSC) reference electrode filled with 3 M KCl. The potentials of the gold electrode sample (U_S) and ECSTM probe (U_P) are expressed against this reference. ECSTM cell and all of the glass material used for preparation of solutions were cleaned with piranha solution (7:3 H_2SO_4/H_2O_2 (30%) by volume). ***Caution: Piranha solution should be handled with extreme caution***. Deionized water (18 MΩ cm^{-1} Milli-Q, Millipore) was used to prepare all solutions and for rinsing samples and electrodes. Solution for experiments was 50 mM ammonium acetate buffer at pH 4.55. ECSTM probes were prepared by cutting a 0.025 mm diameter Au wire (99.99%), briefly flame annealed and isolated with Apiezon wax. In some experiments Pt:Ir probes were prepared using reported methods.[9] Au ECSTM probes were finally used in order to enhance junction symmetry and electronic coupling although no significant differences in conductance histograms were obtained using either material. Data was acquired using a NI-DAQmx and BNC-2110 Labview equipment and analyzed with Origin. Two alternative methods were used to determine single azurin conductance. First, the STM break-junction method (STMBJ)[10] was applied to obtain conductance histograms. Briefly, after bringing the ECSTM probe to tunneling distance to the substrate, the STM feedback loop is turned off and a separate computer is used to move the probe into and out of contact with the

substrate at rates in the 10-40 nm/s range. Current distance curves are collected by these means. Ramps containing plateaus or steps (10% of the cases) can be used to obtain single molecule conductance using $G=I_{step}/U_{bias}$, in a moderate bias range (±0.4V in figure S2). These ramps are used to construct a conductance histogram where peaks represent the conductance value of the observed plateaus in the I(z) traces.[11] This method yields reliable single molecule conductance measurements with high statistics. In order to test the effect of protein redox state on conductance, linear (fig S3) and semilogarithmic histograms (fig. 2 in the manuscript) at different electrochemical gate potential values (different U_S and U_P relative to the reference electrode at constant U_{bias} = -0.3V) were performed, as described elsewhere.[12] In an alternative approach, spontaneously formed single protein junctions were recorded using the current-time STM based method[13]. In this case, after bringing the tip to a tunneling distance to the substrate, the STM feedback is disconnected and the current as function of time is recorded. When a molecule bridges tip and sample electrodes, a sudden "jump" or "blink" in the trace is detected. The magnitude of the blink can be used to find single protein conductance using $G= I_{blink}/U_{bias}$. This method does not offer the possibility of obtaining high statistics but it avoids contact between electrodes and has also the advantage of allowing subsequent electrical measurements once a bridged junction has spontaneously formed. For instance, after a spontaneous single azurin bridge is detected, an automatic algorithm is run in order to ramp the electrochemical gate (relative potential of the reference electrode in the ECSTM configuration, $-U_S$ at constant bias conditions) while the current signal is recorded (Figure 4 in the manuscript and Figure S5 showing Zn-Azurin control experiments). Current-bias voltage characteristics are studied in spontaneously formed bridges by sweeping the ECSTM probe potential (Figure S2). Typically, at the end of the electrical measurement, Z position of the piezo is also swept as in a "pulling" experiment (Figure S4) in order to identify the single-protein junction by the junction breakdown event.

Sequence alignment of protein and peptides.

Multiple sequence alignments were performed using ClustalW2[14, 15] at EMBL-EBI website (http://www.ebi.ac.uk/Tools/msa/clustalw2/) using azurin sequence (Protein Data Bank access code

1AZU)[16] and metal binding peptides reported in the literature[17]. Two regions of the protein display high sequence similarity with the peptides, the first corresponding to the C3-C26 disulfide bridge of azurin (Fig. S1a), in agreement with the reported formation of thiol bonds between these cysteines and metal electrodes.[4, 5] In order to evaluate the zone of interaction with the STM probe, subsequent alignments revealed a different region with high sequence similarity (fig S1b, residues 35 to 48). This zone corresponds to the hydrophobic patch of azurin and contains residue K41, which is exposed to the solvent and charged in the solution used in this experiments.

2. Alignments with peptide binding materials

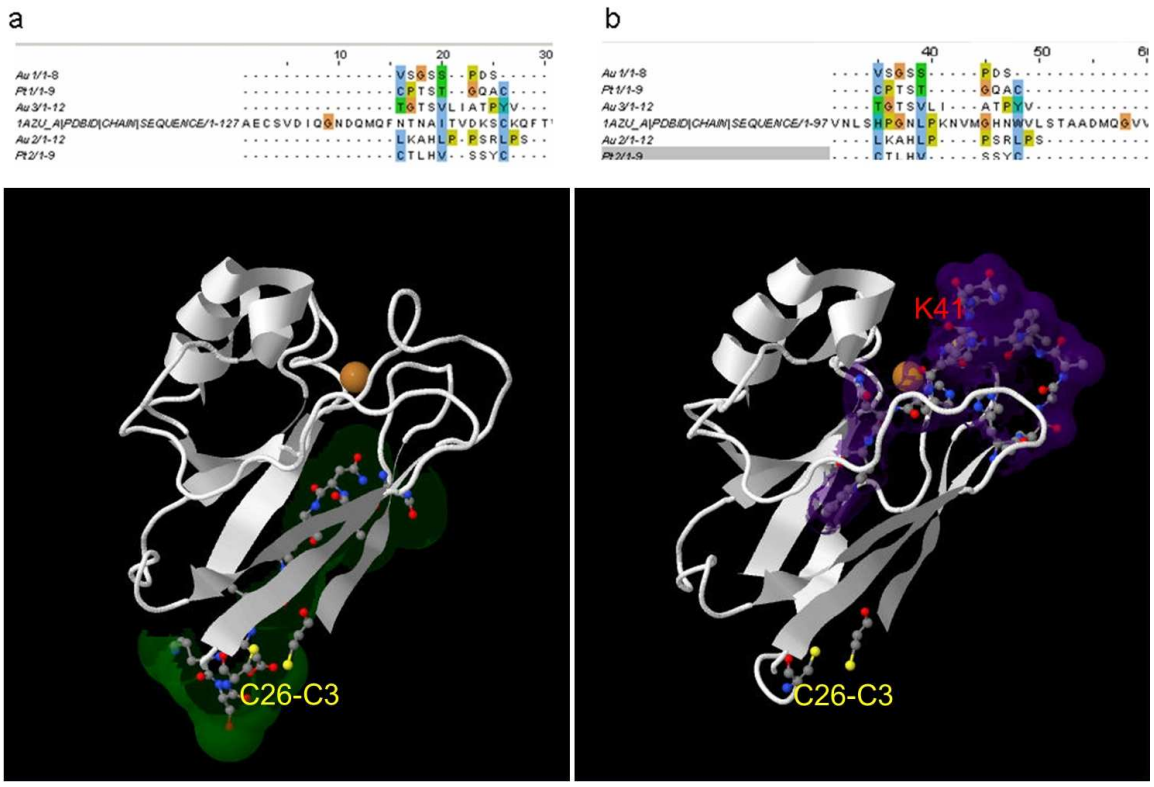

Figure S1. Multiple alignments between metal binding peptides[17] and azurin sequences[16] (top) and 3D schematic representations of azurin structure (1AZU[16]) showing the zones obtained in the alignments. Regions of best alignment are indicated by showing the residues as ball and stick models and their surface accessible to solvent. Best alignment (a) is obtained in the C3-C26 disulfide bridge zone (green surface). Second best alignment (b) is obtained with the zone corresponding to residues 35-48 (violet surface), assuming the first zone in (a) already attached to the gold surface in the ECSTM configuration.[4, 5]

3. Current – Bias Voltage experiments.

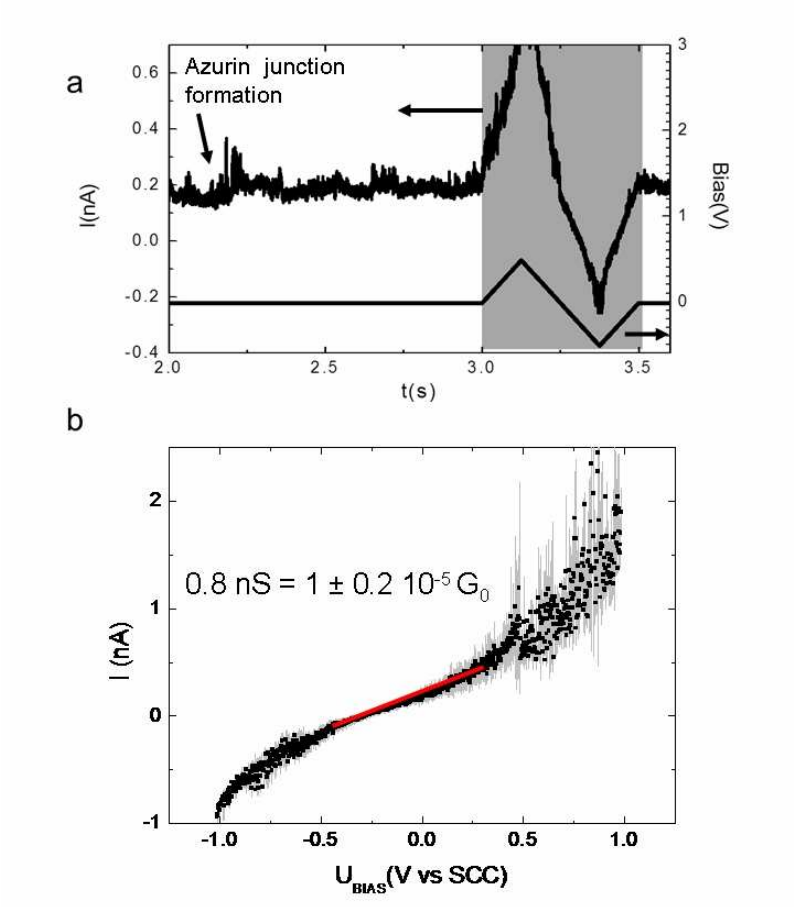

Figure S2. (a) An example of current *vs* time trace (top) where the bias voltage (bottom) in the junction is swept (time interval marked in grey) after spontaneous formation of a single azurin junction (indicated with an arrowhead). Current-time signal refers to the left-bottom axes and U_{bias}-time signal refers to the right-bottom axes, as indicated with the arrows. (b) Black dots indicate the average of 10 current-voltage curves obtained as indicated in (a). A linear fit is shown in red in the ±0.4V range. Error bars (in grey) in the average represent the standard deviation.

The slope of $I(U_{bias})$ plots in the linear region at low bias potentials (Fig. S2) yields a conductance value of $1 \pm 0.2\ 10^{-5}\ G_{0,}$ in very good agreement with the conductance obtained from I(z) histograms (Fig. 1 and 2 in the main text). This fact evidences the formation of a stable single-molecule junction that remains all along the bias potential excursion. Moreover, the asymmetry between the positive and the negative branches of the $I(U_{bias})$ plot at high bias provides a measure of the difference in

electronic coupling between one side of the molecular bridge (where a strong thiol covalently bonds azurin to the gold substrate) and the opposite side (where azurin forms a weaker bond with the Au or Pt STM probe) [18] The current at ±1 V bias in Fig S2 is only a factor 2 higher in the positive branch than in the negative branch of the I(U_{bias}) plot, which corroborates the good electronic coupling achieved between the STM probe and azurin. Similar low asymmetries in I(U) characteristics from molecular junctions have been ascribed to different contact geometries on individual runs[19] and to an asymmetric position of an acceptor group within the molecular backbone,[20] the latter being a similar scenario to our single-azurin junction where the Cu(I) center is asymmetrically located versus both electrodes at the junction.[16]

4. Linear histograms at different substrate potentials.

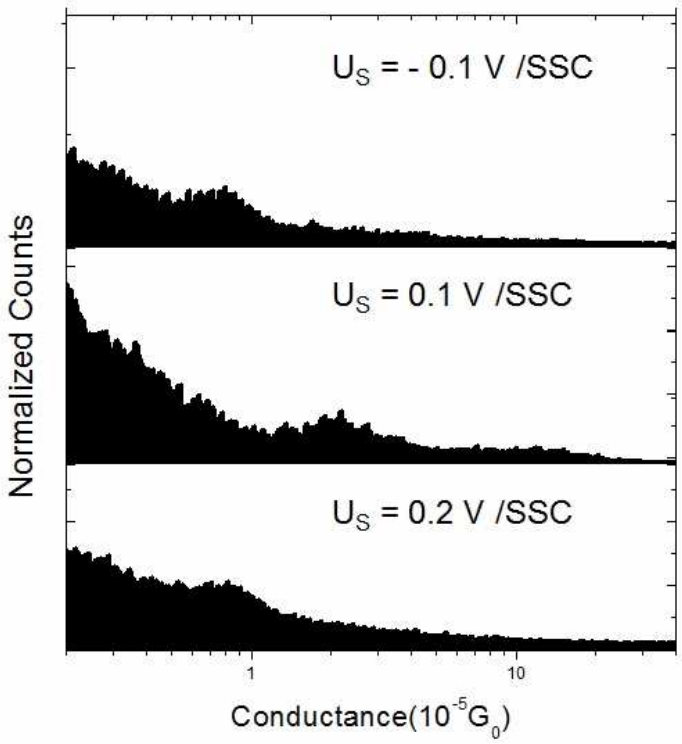

Figure S3. Semilogarithmic representation of linear conductance histograms at different U_S potentials (indicated in each panel). These histograms correspond to the logarithmic histograms shown in fig. 2 in the manuscript.

5. Single protein junction pulling experiments.

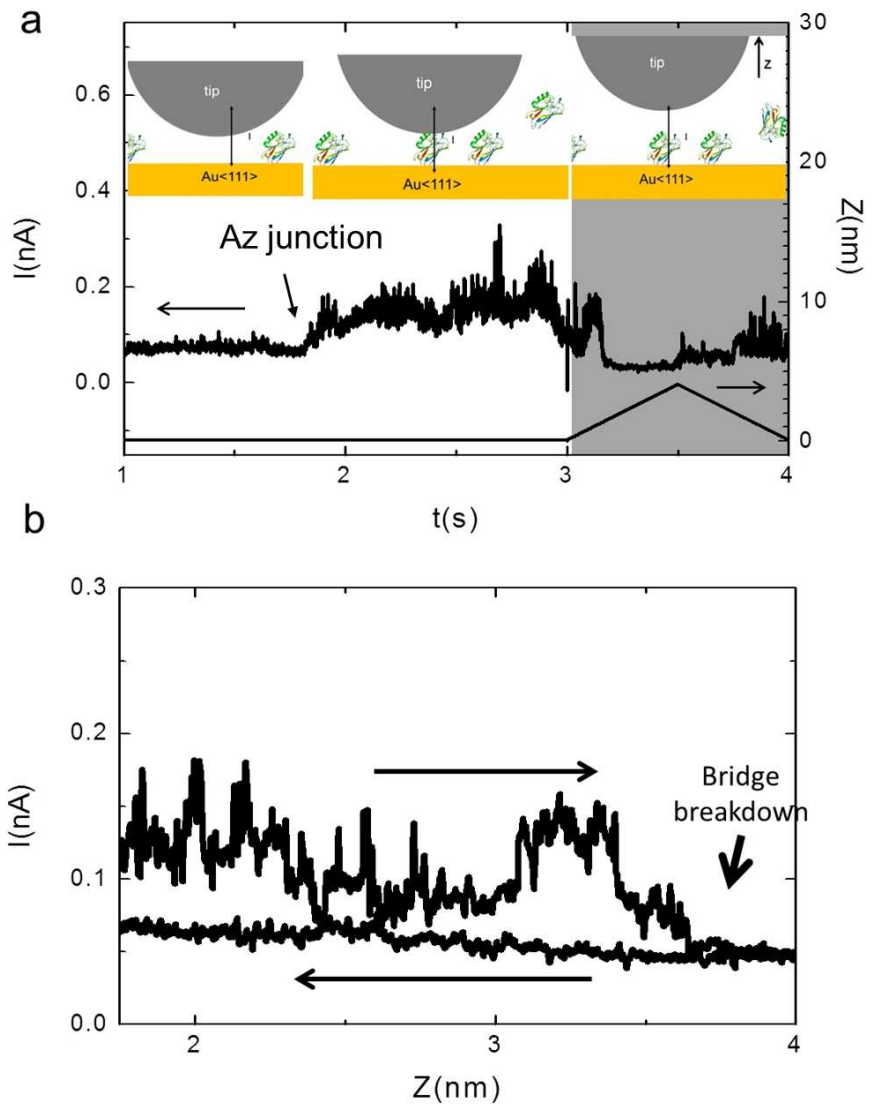

Figure S4. (a) An example of current *vs* time trace (top) where the Z position of the piezo is swept (time interval marked in grey) after spontaneous formation of a single azurin junction (indicated with an arrowhead). Current-time signal refers to the left-bottom axes and Z-time signal refers to the right- bottom axes, as indicated with the arrows. (b) Current distance trace of a typical pulling experiment showing a bridge breakdown after a certain distance is retracted.

6. Non-redox Zn Azurin EC gate control experiments.

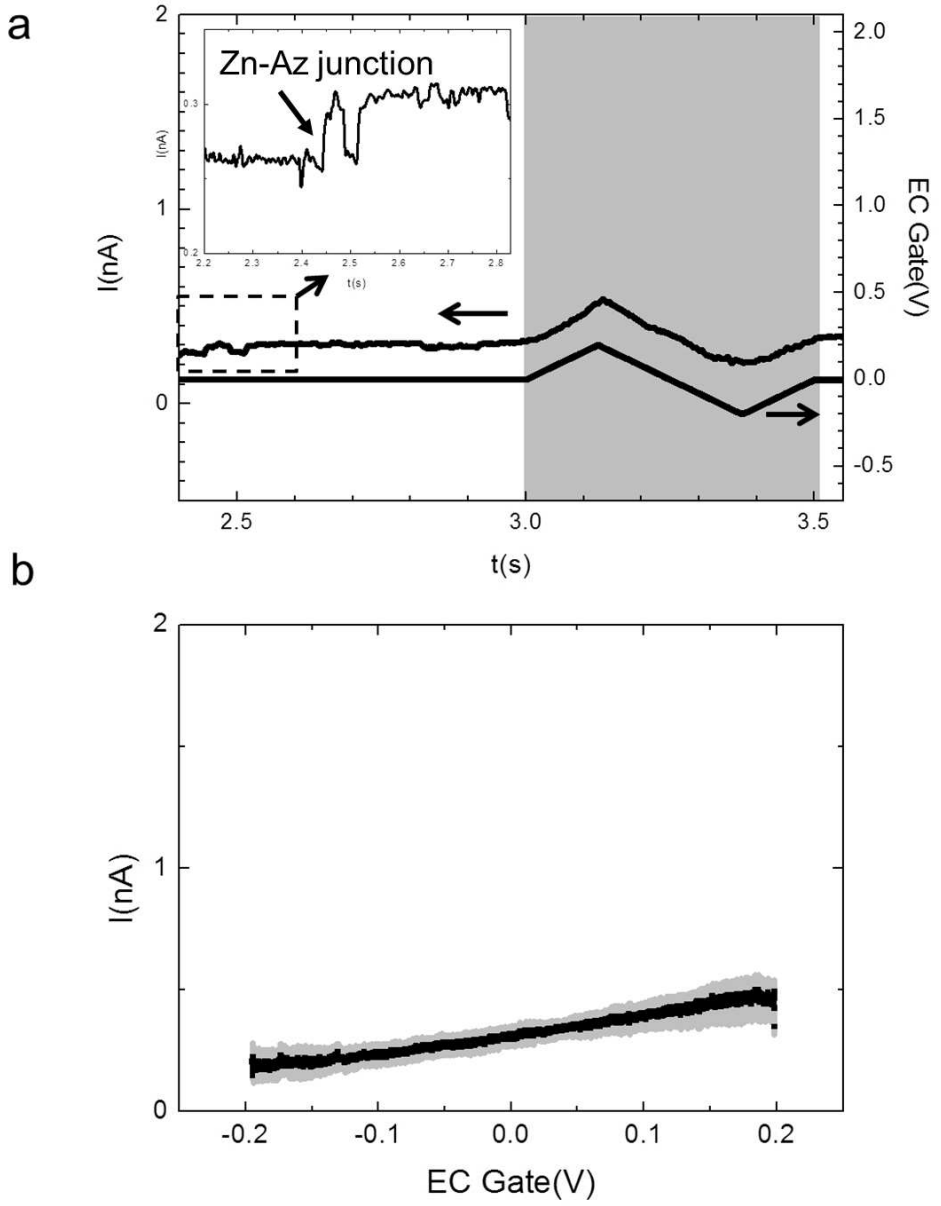

Figure S5. (a) An example of current *vs* time trace (top-left axes) where the EC gate potential (bottom-right axes) in the junction is swept (time interval marked in grey) after spontaneous formation of a single Zn-azurin junction (indicated with an arrowhead). Current/time signal refers to the left-bottom axes while EC Gate/time signal refers to the right-bottom axes. Inset is a zoom of the small "blink" obtained for non-redox Zn-azurin. (b) Average of N=10 current-EC gate potential curves obtained in non-redox Zn-azurin control experiments. Error bars (in grey) in the average represent the standard deviation.

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